aliquot of [the] a stable enzyme composition [of Claim 1] comprising a purified thermostable nucleic acid polymerase enzyme in a buffer and further comprising one or more nonionic polymeric detergents.

Remarks

The Invention

The present invention provides stabilized thermostable nucleic acid polymerase compositions. Since 1987 and due to the benefits provided by the present application, thermostable nucleic acid polymerases have come into widespread use. Suppliers that formulate their thermostable nucleic acid polymerase with the stabilizers of the present invention have met with great commercial success. These stabilizers, non-ionic polymeric detergents, help maintain polymerase activity in purified preparations of thermostable nucleic acid polymerase during the heating and cooling steps of the PCR nucleic acid amplification process.

The Amendments to the Specification

Applicants have requested an amendment to correct a typographical error on page 5.

The Amendments to the Claims

Applicants have requested a number of amendments to the claims to address the concerns expressed by Examiner in the rejection under 35 U.S.C. §112, as discussed below.

The Rejection Under 35 U.S.C. §112

A. First Paragraph Rejections

Examiner has objected to the specification and rejected a number of claims under the first paragraph of 35 U.S.C. §112 for a number of different reasons. To facilitate discussion, each different reason and the set of claims to which the reason applies is discussed separately below.

91

1. Claims 56-61

Examiner has objected to the specification and rejected Claims 56-61, stating:

> The specification fails to support the detergent being present in an amount of 0.1% to 1.0% as required by claim 56. While there is disclosure of 0.1% to 0.5% for one or more detergents and original claims 38 and 39 require two detergents, this does not support that 1.0% was to be an upper limit. When two detergents are used, the amount could have been double any amount below 05.% rather than double the upper limit of 0.5%. Moreover, when a plurality of detergents are used, the amount could be the same as when only one detergent is used.

The specification does support the range of detergent concentrations found in Claims 56-61.

In Example XIV, page 79, lines 8-10, Applicants state:

> In addition, the Taq polymerase is preferably stored in storage buffer containing from about 0.1 to 0.5% volume/volume of each non-ionic polymeric detergent employed.

In the immediately following text, Applicants describe a preferred buffer and explicitly support the upper end of the range of Claim 56. Examiner will note that the detergents in the preferred buffer are listed on line 13 of page 79: "0.5% NP-40, 0.5% v/v Tween 20 . . . ." The specification therefore supports the detergent concentration range in Claims 56-61, and Applicants accordingly request that the rejection be withdrawn.

2. Claim 62

Examiner has objected to the specification and rejected Claim 62, because:

> While a mixture as claimed may be formed when a reaction is carried out, this mixture exists only for a moment since the reaction begins as soon as the mixture is formed. There is inadequate support for a mixture that exists momentarily during a reaction as part of the invention.

The specification does support the reaction mixture of Claim 62, which is not a "mixture that exists momentarily."

In Example XIV, page 79, lines 17-22, Applicants describe a reaction mixture of Claim 62. Examiner will note from this description that the reaction mixture does not include template. Without template, the reaction mixture does not exist "only for a moment," because the reaction requires template nucleic acid. Claim 62 does not require template nucleic acid in the mixture.

Yet even when template is added, as described at page 79, lines 22-24, the reaction does not begin until the template is rendered single-stranded. If double-stranded DNA is the template, the reaction requires an initial denaturation step, typically a heating step, to begin. Thus, even a

reaction mixture with template, within the scope of Claim 62, can exist longer than "only for a moment."

Finally, even when the reaction is underway, and the repeated steps of primer annealing and extending and template denaturing are being conducted, the reaction mixture contains the elements of Claim 62. The detergents, polymerase, and buffer are not consumed, and the primers and nucleoside triphosphates must be present for the reaction to occur.

Even if the reaction mixture "exists only for a moment," Applicants have shown how to make that mixture in Example XIV. The patent law does not describe inventions patentable in terms of how long the invention exists once made. Applicants accordingly request Examiner to withdraw the rejection.

3. Claims 1, 35-39, and 53-62

Examiner rejected Claims 1, 35-39, and 53-62, stating:

> [T]he disclosure is enabling only for claims limited to a buffer which contains substances in addition to the detergent as disclosed in the preferred embodiment. There is inadequate support that only a detergent will provide an operable buffer.

Applicants have amended Claim 1 to address Examiner's concern.

Applicants never intended to describe the stabilizing detergents as buffers. Nor is the particular buffer used an essential or critical feature of the invention. Thus, Applicants intend Claim 1 to describe a "thermostable nucleic acid polymerase enzyme in a buffer and further comprising" a non-ionic detergent as a stabilizer. Claim 1 has been amended to reflect Applicants' intent.

Applicants request Examiner to withdraw the rejection in view of the amendment to Claim 1.

B. Second Paragraph

1. Claim 38

Examiner made a number of suggestions to improve the clarity of Claim 38. Applicants have amended the claim as suggested by Examiner and so request withdrawal of the rejection.

2. Claims 57-61

Examiner rejected Claims 57-61, because of the phrase "Thermus species." Examiner correctly points out that Thermus is a genus. Applicants believe Claim 57 does not suggest that

Thermus is a species but, to address Examiner's concern, have amended the claim. The claim now recites "a species of the genus Thermus" in place of "Thermus species." Applicants request that the rejection be withdrawn in view of the amendment.

3. Claim 62

Examiner rejected Claim 62 under the second paragraph of 35 U.S.C. §112 for the same reason that claim was rejected under the first paragraph of 35 U.S.C. §112. Applicants have addressed Examiner's concern and request withdrawal of this rejection for the same reasons.

C. Fourth Paragraph

Examiner has rejected Claim 62 under the fourth paragraph of 35 U.S.C. §112 as "being of improper dependent form for failing to further limit the subject matter of a previous claim." Examiner does not believe the reaction mixture of Claim 62 further limits the composition of Claim 1.

Applicants appreciate Examiner's concern and have rewritten Claim 62 in independent form. Amended, independent Claim 62 contains all of the limitations of former Claim 62 and Claim 1. Examiner is respectfully requested to withdraw the rejection of Claim 62 in view of this amendment.

The Rejection Under 35 U.S.C. §102(a)

The Examiner has rejected Claims 1, 35-39, and 53-59 under 35 U.S.C. §102(a) "as being anticipated by the MBR product information referred to in the last paragraph of the letter of Staple of 6/9/87" (copy supplied of information sheet entitled "DNA Polymerase"). Examiner also reiterates Applicants' assertion that MBR derived the present invention from Applicants. Apparently, however, Examiner places more weight on MBR's counter-assertions that "the MBR product was developed independent of the information provided by applicants."

In the accompanying Declaration Under 37 C.F.R. §1.131, inventor David Gelfand states that the stabilized enzyme composition of the instant claims was reduced to practice before May 1, 1987, well before the date of receipt of the MBR information sheet referred to in the Staple letter.

In addition, the Declaration shows that Applicants believe MBR derived the invention from the present inventors. The letter and protocol attached to the Declaration show that Cetus supplied

information to MBR disclosing the present invention to MBR well prior to the public disclosure of that information by MBR.

In view of the accompanying Declaration, Examiner is respectfully requested to reconsider and withdraw the rejection under 35 U.S.C. §102(a).

The Rejection Under 35 U.S.C. §103

Examiner rejected Claims 40, 41, and 60-62 under 35 U.S.C. §103 as being unpatentable over the product information of MBR. Applicants respectfully request Examiner to reconsider and withdraw the rejection in view of the accompanying Declaration Under 37 C.F.R. §1.131, for the reasons discussed above.

Examiner rejected Claims 1, 35-41, 53-59, and 62 under 35 U.S.C. §103 as being unpatentable over Kaledin et al. (1980) in view of Goff et al., Feller et al., or Spiegelman. Examiner's reasoning is as follows:

> It would have been obvious to store the polymerase of Kaledin et al. in a buffer containing a nonionic detergent in view of Goff et al. disclosing (col. 8, line 24) that a nonionic detergent is required in recovering this enzyme and if needed in further view of Feller et al. (col. 5, line 7) or Spiegelman (col. 6, line 25) disclosing use of a detergent-containing buffer in relation to this type of enzyme.

Examiner then rejected Claims 60 and 61 under 35 U.S.C. §103 using the same reasoning but different primary references: Kaledin et al. (1981) and Ruttiman et al. (1985).

The primary references cited by Examiner each describe attempts to purify a thermostable DNA polymerase. The Kaledin et al. (1980) reference relates to Thermus aquaticus; the Kaledin et al. (1981) reference to T. flavus; and the Ruttiman et al. (1985) to T. thermophilus. None of the primary references, however, mentions the improved stability of preparations with non-ionic detergents or suggests any formulations of a thermostable nucleic acid polymerase with non-ionic detergents.

Applicants' claims are limited to stabilized thermostable nucleic acid polymerases. The primary references do not suggest any reason to change the formulations described in the references. The authors of the Kaledin et al. (1980) reference believed that they had solved any problem of stability. On page 497 of that reference, the authors state:

> The introduction of gelatin stabilizes the enzymatic activity. If gelatin is not introduced in this stage, the enzyme quickly loses its activity. DNA polymerase is also stabilized by albumin, but when it is used, it is difficult to determine the specific activity at 70°C.

Having supposedly solved the problem of stability, the authors of the Kaledin et al. (1980) reference did not suggest any alternatives, other than gelatin or albumin, to the stabilizers present in their thermostable nucleic acid preparations. Having read the reference, one of ordinary skill would not be motivated to change the formulations of the reference so as to arrive at the present invention.

The authors of the Kaledin et al. (1981) article also addressed stability of their enzyme preparation. At page 1250 of the reference, the authors state:

> After heparin-Sepharose 4B, the enzyme preparation is very labile in view of the low protein concentration in it (about 70 μg/ml); therefore, we stabilized it with autoclaved gelatin in a concentration of 250 μg/ml).

However, the authors apparently did not believe stability was a problem with regard to their final preparation, which was stored in buffer C with 50% glycerol (see section five on page 1250). The final preparation apparently did not contain gelatin, but the authors report that that preparation "was stored at -20°C without appreciable loss of activity for at least three years." Id. Once again, to the extent the authors of the Kaledin et al. (1981) reference recognized a problem with stability, the authors reported that the problem was solved by the addition of gelatin. The reference offers no reason or motivation for one of ordinary skill in the art to change the formulation of the reference to overcome the problem of stability.

The authors of the Ruttiman et al. (1985) reference likewise offer no motivation for one of ordinary skill in the art to alter the formulation of their polymerase. On page 44 of the Ruttiman et al. (1985) reference, the authors state:

> When the purification processes were carried out at room temperature, similar results were obtained, since the enzymes were stable for one or two weeks at 20°C. The final fractions have retained their activity over a 6-month period stored at -20°C, although only pol B and C resist repeated freezing and thawing.

The authors stored their purified polymerase fractions in buffer A (see top of right hand column of page 43, section entitled "Concentration of the Enzymes"), and buffer A does not contain a detergent (see page 42, right hand column, section entitled "Purification of DNA Polymerases"). Because the authors of the Ruttiman et al. (1985) reference do not describe a stability problem with their formulations of thermostable nucleic acid polymerase, the reference does not motivate one of ordinary skill to alter the formulations so as to arrive at the present invention.

Examiner's rejection under 35 U.S.C. §103 presupposes that one of ordinary skill, upon reading the primary references, would be motivated to change the formulations the thermostable

enzyme preparations disclosed in those references so as to arrive at Applicants' invention. The law is clear; an obviousness rejection cannot be maintained where "the prior art of record provides one skilled in the art no motivation to make the molecular modification needed to arrive at . . . [Applicants' invention]." In re Taborsky, 183 U.S.P.Q. 50, 55 (C.C.P.A. 1974) (emphasis added); accord In re Lintner, 173 U.S.P.Q. 560, 562 (C.C.P.A. 1972). However, as shown above, a review of the primary references cited by Examiner reveals no reason one of ordinary skill in art would change the formulations described in those references so as to arrive at Applicants' invention.

Applicants respectfully assert, therefore, that the Examiner's rejection is improper. Applicants believe the law fully supports this position:

> In determining the propriety of the Patent Office case for obviousness . . . it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the references before him to make the proposed substitution, combination, or other modification.

In re Lintner, 173 U.S.P.Q. 560, 562 (C.C.P.A. 1972) (emphasis added). Having reviewed the secondary references cited by Examiner, Applicants believe that, at most, Examiner has mechanically combined brief statements in the secondary references with the teaching of the primary references to support the obviousness rejection. Such combination, however, is improper without some showing that the prior art would motivate one of ordinary skill to change or modify the teachings of the primary references:

> While, as an abstract proposition, it might be possible to select certain statements from . . . [two references] and mechanically combine them . . . we find absolutely no basis for making such a combination . . . [when it is] only appellant's specification [that] suggests any reason for combining the teachings of the prior art . . . use of such suggestion is, of course, improper under the mandate of 35 U.S.C. 103.

In re Pye and Peterson, 148 U.S.P.Q. 426, 429 (C.C.P.A. 1966).

The three secondary references cited by Examiner and discussed more fully below do not teach stabilized thermostable nucleic acid polymerase enzyme formulations containing nonionic detergents. None of the secondary references in any way suggests that nonionic detergent be present in purified thermostable nucleic acid polymerase preparations.

Examiner points to three lines of the Goff et al. '531 patent as teaching that a nonionic detergent is required in recovering the reverse transcriptase enzyme described in the patent. These three lines, at column 8, lines 23-25, are "[r]ecovery of the activity in the soluble fraction required

the presence of nonionic detergent and high salt concentrations (data not shown)." These few sentences merely show that the inventors of the '531 patent put nonionic detergent in the crude extracts prepared from lysing cells to prevent the reverse transcriptase from associating with the cellular debris. The storage buffer described at column 13, lines 62-64, also contains nonionic detergent, and at column 20, lines 24-26, the inventors state that the "presence of a nonionic detergent was required throughout the purification to prevent aggregation and loss of activity."

The patent does not suggest that stability problems associated with thermostable nucleic acid polymerases could similarly be solved by the use of nonionic detergents. Instead, the patent focuses exclusively on recombinant murine Maloney leukemia virus reverse transcriptase enzymes and offers no motivation for applying the teachings relating to those enzymes to the purification and storage stability of thermostable nucleic acid polymerase preparations.

Applicants respectfully assert that Examiner uses hindsight in combining the primary and secondary references. As evidence of this hindsight, Applicants provide herewith selected pages from the New England Biolabs 1988-1989 Catalog. On pages 45-47 of this catalog, copies enclosed, Examiner will find reference to seven different nucleic acid polymerases, only one of which is thermostable. Under the heading "Concentration and Shipping," Examiner should note the buffer in which each different polymerase enzyme is supplied. Examiner will find that only the murine Maloney leukemia virus reverse transcriptase is supplied in a buffer that contains nonionic detergent. The other polymerases are not shipped in buffer that contains nonionic detergent. Instead, these polymerases are shipped in buffers that comprise 50% glycerol. The fact that only one out of seven of the polymerases sold contains nonionic detergent in the shipping buffer certainly does not support the contention that one of ordinary skill would routinely use nonionic detergent in a thermostable polymerase preparation. Applicants respectfully assert that the only motivation for combining the teaching of the primary references with the teaching of the secondary references comes only from Applicants' specification. Therefore, the rejection under 35 U.S.C. §103 should not stand.

Turning to the other secondary references cited by Examiner, Applicants first note that both the Feller et al. '200 patent and the Spiegelman et al. '839 patent both relate to assays for reverse transcriptase activity. Applicants believe that both references describe the use of nonionic detergent merely to lyse cells or to permeabilize cells and viral particles to allow for the highly charged dioxyribonucleoside triphosphates used in the assay to make contact with the reverse transcriptase

present in the sample being assayed. Neither reference suggests or discloses that the nonionic detergent serves any stabilizing or activity enhancing purpose.

The Feller et al. '200 patent describes a process for purifying reverse transcriptase from human milk. At column 5, lines 6-8, the patent discloses that an appropriate buffer for the enzyme contains 0.2% detergent. Example 1 of the patent, at column 15, line 28, shows that NP40 is one such detergent. The patent does not teach that a nonionic detergent would be useful in stabilizing thermostable nucleic acid polymerases or stress the importance of the detergent to the stability of the human reverse transcriptase described.

The Spiegelman '839 patent describes a method for detecting cancer that involves assaying for reverse transcriptase activity associated with tumor causing viruses. In Example 1, at page 6, lines 17-29, the inventors describe a buffer for disrupting cells in the first stages of a subcellular fractionation prior to a polymerase assay. It is unclear whether the nonionic detergent is actually present in the final preparation of the enzyme, because the authors do not state the buffer in which the final concentration step is performed (column 6, lines 46-49). Even if this concentration buffer is buffer A, which contains 0.02% Triton X-100, however, the patent still does not offer any teaching or suggestion that would motivate one of ordinary skill to use this detergent in preparing stabilized preparations of a thermostable nucleic acid polymerase. Without this motivation or teaching, the Spiegelman '839 patent does not provide the necessary teaching to support the Examiner's rejection under 35 U.S.C. §103.

The secondary references cited by Examiner deal exclusively with the preparation of non-thermostable reverse transcriptase enzymes. There is no teaching in any of the secondary references that would motivate one of ordinary skill to apply the teachings of the secondary references to the purification of thermostable nucleic acid polymerases. This is especially true in view of the primary references cited by Examiner, which, to the extent the stability problem is addressed at all, purport to solve the stability problem by the addition of gelatin or albumin. There is no teaching in either the primary or secondary references that the nonionic detergent discussed in the secondary references is in any way equivalent to the gelatin or albumin suggested in the primary references.

Applicants therefore respectfully request Examiner to withdraw the rejection of Claims 1, 35-41, 53-59, and 62 under 35 U.S.C. §103.

Respectfully submitted,

By: _____
Kevin R. Kaster
Attorney for Applicants
Reg. No. 32,704

February 8, 1991

CETUS CORPORATION
1400 Fifty-Third Street
Emeryville, CA 94608
(415) 420-3444